

CLAIMS

We claim:

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1. A method of detecting the presence of *Pneumocystis carinii* in a biological specimen, comprising:
amplifying a highly conserved region within a human-*P. carinii* nucleic acid sequence, if such sequence is present in the sample, using two or more oligonucleotide primers derived from human-*P. carinii* MSG protein encoding sequence; and
determining whether an amplified sequence is present.
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2. The method according to claim 1, wherein amplification of the human-*P. carinii* nucleic acid sequence is by polymerase chain reaction.
3. The method of claim 1, wherein the human-*P. carinii* nucleic acid sequence is a highly conserved region within an MSG-protein encoding sequence.
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4. The method of claim 3, wherein the highly conserved region comprises a sequence selected from the group consisting of: residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).
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5. The method of claim 1, wherein at least one oligonucleotide primer comprises at least 15 contiguous nucleotides from a sequence chosen from the group consisting of: residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15) and nucleic acid sequences having at least 70% sequence homology with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).
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6. The method of claim 5, wherein at least one oligonucleotide primer comprises at least 15 contiguous nucleotides from a nucleic acid sequence having at least 90% sequence homology with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).
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7. The method of claim 5, wherein at least one oligonucleotide primer comprises at least 15 contiguous nucleotides from a nucleic acid sequence having at least 95% sequence homology with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of

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 5 *523* HMSG32 (SEQ ID NO: 9), 2887-3132 of HMSG33 (SEQ ID NO: 11), 2821-3072 of HMSG35 (SEQ ID NO: 13), and 1-249 of HMSGp2 (SEQ ID NO: 15).

8. The method of claim 5, wherein the oligonucleotide primers are chosen from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 23, and SEQ ID NO: 24.

9. The method of claim 5, wherein the pair of oligonucleotide primers consist of one upstream primer and one downstream primer.

10. The method of claim 9, wherein:
 the upstream primer is chosen from the group consisting of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 23; and
 the downstream primer is chosen from the group consisting of: SEQ ID NO: 20 and SEQ ID NO: 24.

11. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 17.

12. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 18.

13. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 19.

14. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 20.

15. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 23.

16. The method of claim 8, wherein one of the oligonucleotide primers comprises SEQ ID NO: 24.

17. The method of claim 1, wherein the biological specimen is from the oropharyngeal tract.

18. The method of claim 1, wherein the biological specimen is from blood.

19. The method of claim 1, wherein the step of determining whether an amplified sequence is present comprises one or more of:

- (a) electrophoresis and staining of the amplified sequence; or
- (b) hybridization to a labeled probe of the amplified sequence.

20. The method of claim 19, wherein the amplified sequence is detected by hybridization to a labeled probe.

21. The method of claim 22, wherein the probe comprises a detectable non-isotopic label chosen from the group consisting of:

- a fluorescent molecule;
- a chemiluminescent molecule;
- an enzyme;

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a co-factor;
an enzyme substrate; and
a hapten.

22. The method of claim 21, wherein the labeled probe comprises a nucleic acid sequence according to SEQ ID NO: 19.

23. A method of detecting the presence of *Pneumocystis carinii* in a biological specimen, comprising:
exposing the biological specimen to a probe that hybridizes to a highly conserved region within a human-*P. carinii* nucleic acid sequence, if the sequence is present in the sample to form a hybridization complex; and
determining whether the hybridization complex is present wherein the nucleic acid sequence derived from human-*P. carinii* is an MSG encoding sequence.

24. The method of claim 23, wherein the labeled probe comprises a nucleic acid sequence according to SEQ ID NO: 19.

25. A purified protein comprising an amino acid sequence selected from the group consisting of

- (a) SEQ ID NO: 2;
- (b) SEQ ID NO: 4;
- (c) SEQ ID NO: 6;
- (d) SEQ ID NO: 8;
- (e) SEQ ID NO: 10;
- (f) SEQ ID NO: 12;
- (g) SEQ ID NO: 14;

and conservative substitutions thereof.

26. An isolated nucleic acid molecule encoding a protein according to claim 25.

27. The isolated nucleic acid molecule according to claim 26, wherein the nucleic acid molecule has a sequence selected from the group consisting of: SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 15; and SEQ ID NO: 17.

28. An isolated nucleic acid molecule comprising a sequence selected from the group consisting of: residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15); and a sequence with at least 70% sequence identity with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ

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ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

29. An isolated nucleic acid molecule comprising a sequence selected from the group consisting of: at least 15 contiguous nucleotides of the nucleic acid molecule according to claim 28.

30. An isolated nucleic acid molecule comprising a sequence selected from the group consisting of: at least 20 contiguous nucleotides of the nucleic acid molecule according to claim 29.

31. A recombinant vector comprising the nucleic acid molecule according to claim 28.

32. A transgenic cell comprising the vector according to claim 31.

33. A kit for detecting a human-*P. carinii* nucleic acid sequence comprising at least a pair of primers each comprising at least 15 contiguous nucleotides of sequence selected from the group consisting of: residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15); and a sequence with at least 70% sequence identity with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

34. A kit for detecting a human-*P. carinii* nucleic acid sequence comprising at least a pair of primers each comprising at least 20 contiguous nucleotides of sequence selected from the group consisting of: residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15); and a sequence with at least 70% sequence identity with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

35. A kit for detecting a human-*P. carinii* nucleic acid sequence comprising at least a pair of primers each comprising at least 30 contiguous nucleotides of sequence selected from the group consisting of: residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15); and a sequence with at least 70% sequence identity with residues 2894-3042 of *HMSGp1* (SEQ ID NO: 1), 2758-3006 of *HMSGp3* (SEQ ID NO: 3), 2845-3090 of *HMSG11* (SEQ ID NO: 5), 2839-3084 of *HMSG14* (SEQ ID NO: 7), 2836-3081 of *HMSG32* (SEQ ID NO: 9), 2887-3132 of *HMSG33* (SEQ ID NO: 11), 2821-3072 of *HMSG35* (SEQ ID NO: 13), and 1-249 of *HMSGp2* (SEQ ID NO: 15).

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36. The kit of claim 33, wherein at least one of the oligonucleotide primers comprises a sequence selected from the group consisting of: SEQ ID NO: 17; SEQ ID NO: 18; SEQ ID NO: 19; SEQ ID NO: 20; SEQ ID NO: 21; SEQ ID NO: 22; SEQ ID NO: 23; and SEQ ID NO: 24.

37. The kit of claim 36, wherein one of the oligonucleotide primers comprises the sequence according to SEQ ID NO: 17.

38. The kit of claim 36, wherein one of the oligonucleotide primers comprises the sequence according to SEQ ID NO: 18.

39. The kit of claim 36, wherein one of the oligonucleotide primers comprises the sequence according to SEQ ID NO: 19.

40. The kit of claim 36, wherein one of the oligonucleotide primers comprises the sequence according to SEQ ID NO: 21.

41. The kit of claim 36, wherein one of the oligonucleotide primers comprises the sequence according to SEQ ID NO: 22.

42. The kit of claim 36, wherein one of the oligonucleotide primers comprises the sequence according to SEQ ID NO: 23.

43. The kit of claim 36, wherein one of the oligonucleotide primers comprises the sequence according to SEQ ID NO: 24.

44. Antibody raised against the peptide sequence according to SEQ ID NO: 25.

45. Antibody raised against the peptide sequence according to SEQ ID NO: 26.

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